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Tissue uptake of radioactive cholesterol in the prawn *Penaeus japonicus* Bate during induced ovarian maturation

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Abstract

The *in vivo* distribution and fate of ^{14}C-cholesterol injected in prepuberal female prawns, *Penaeus japonicus* Bate, intact (E^+) or deprived of their eyestalks (E^-), were investigated. Animals were injected at the beginning of the experiment. Three days later, half of them (24 specimens) supported bilateral eyestalks ablation within a 48 hours period. Four days after the ablation, the ovaries of eyestalkless animals were relatively more developed than those of intact animals as seen by the increase in ovarian weight. The total content ^{14}C-cholesterol and/or its metabolic products (total dpm) in whole animals was not significantly different between the two lots of animals and remained nearly constant throughout the experiment.

Distribution of radioactivity (dpm/mg wet weight of tissue) in carcass, muscle, gut, hepatopancreas, eyes and eyestalks and ovaries from E^+ and E^- after 192 hours of ^{14}C-cholesterol injection indicate that ovaries presented the highest concentration of label (6 to 40 times higher than other organs) while these differences were not statistically significant between E^+ and E^-. The study of incorporation of ^{14}C-cholesterol in those organs from E^+ and E^- independent of their volume, expressed as percentage organ radioactivity/whole animal radioactivity, at different time points, indicate that major accumulation was achieved by the carcass and muscle although radioactivity in the muscle progressively increased in inverse correlation with the decrease observed in the carcass. Hepatopancreas, eyes and eyestalks, gut and ovaries showed small incorporation that was kept constant with time in eyes and guts and significantly decreased in ovaries and hepatopancreas. At 192 hours after ^{14}C-cholesterol injection there was an apparent increase in ^{14}C label retention in ovaries from E^- compared to E^+ that was not statistically significant.

Results indicate that the ovary is presumably the major site of cholesterol metabolism followed by the hepatopancreas. Muscle seemingly is the major site for storage of large amounts of cholesterol and/or its metabolites while eyes and gut have negligeable retention. Furthermore, in our experimental conditions, the requirement for cholesterol in different tissues does not vary significantly with eyestalk ablation suggesting that the phenomenon could be independent of ovarian maturation and moulting.

Keywords : Radioactive cholesterol, *Penaeus japonicus*, ovarian maturation.

Quantité de cholestérol radioactif fixé par les tissus chez la crevette Penaeus japonicus *Bate, durant l'induction de la maturation ovarienne.*

Résumé

La localisation et l'évolution de ^{14}C-cholestérol ont été étudiées chez des femelles prépubères de *Penaeus japonicus* Bate, intactes (E^+) ou épédonculées bilatéralement (E^-). Les animaux ont subi une injection au début de l'expérience. Trois jours après l'injection, la moitié d'entre eux (24 individus) a subi l'ablation bilatérale des pédoncules oculaires sur une période de 48 heures. Quatre jours après l'ablation des pédoncules, les ovaires des animaux épédonculés étaient relativement plus développés que ceux des animaux intacts, comme le montre l'accroissement du poids des ovaires. Le contenu

total de ^{14}C-cholestérol et/ou des produits de son métabolisme (dpm totale) n'est pas significativement différent entre les animaux intacts et les animaux épédonculés et demeure à peu près constant pendant toute la durée de l'expérience.

La distribution de la radioactivité (dpm total/mg de poids frais de tissu) dans la carcasse, le muscle, l'intestin, l'hépatopancréas, les yeux et les pédoncules oculaires et les ovaires des animaux E^+ et E^-, 192 heures après l'injection de ^{14}C-cholestérol, montre que les ovaires contiennent la concentration la plus élevée de radiotraceur (6 à 40 fois plus que dans les autres organes), les différences entre les animaux E^+ et E^- n'étant toutefois pas statistiquement significatives. L'étude de l'incorporation de ^{14}C-cholestérol dans ces organes chez les animaux E^+ et E^-, indépendamment de leur volume, exprimée comme le pourcentage de radioactivité dans l'organe par rapport à la radioactivité totale de l'animal, à différents intervalles de temps, indique que l'accumulation a principalement lieu dans la carcasse et dans le muscle, quoique la radioactivité dans le muscle augmente au cours du temps de manière inversement proportionnelle à la diminution observée dans la carcasse. L'hépatopancréas, les yeux et les pédoncules oculaires, l'intestin et les ovaires montrent une incorporation faible qui reste constante au cours du temps dans les yeux et l'intestin et décroît de manière significative dans les ovaires et l'hépatopancréas. 192 heures après l'injection de ^{14}C-cholestérol, les ovaires des femelles E^-, comparés à ceux des femelles E^+, montrent une augmentation apparente de rétention du radiotraceur statistiquement non significative.

Les résultats montrent que l'ovaire est vraisemblablement le siège principal du métabolisme du cholestérol, suivi par l'hépatopancréas. Le muscle est apparemment le site principal de dépôt de grandes quantités de cholestérol et/ou des produits de son métabolisme, alors que les yeux et le pédoncule oculaire et l'intestin ont un rôle négligeable. De plus, dans nos conditions expérimentales, la demande en cholestérol des différents tissus ne varie pas de manière significative avec l'épédonculation, ce qui suggère que le phénomène serait indépendant de la maturation ovarienne et de la mue.

Mots-clés : ^{14}C-cholestérol, *Penaeus japonicus*, maturation ovarienne.

INTRODUCTION

Since the study of Hudinaga and Miyamura (1962) the techniques for mass production of larvae of the prawn *Penaeus japonicus* were established initially in Japan. Nevertheless, the major impediment for the complete culture from hatching to spawning of this species has been the inability to obtain ovarian maturation and spawning with animals held in captivity. In order to solve this problem, Laubier-Bonichon (1975) and then Kanazawa (1982) have studied and succeeded in obtaining spawning of *P. japonicus*, principally by controlling photoperiod and water temperature. For a better understanding of the mechanism of the reproduction of penaeid prawns, a complete histological study of the ovary at different steps of sexual development of animals was carried out (Laubier *et al.*, in prep.).

Similarly to other crustaceans studied (Meusy and Charniaux-Cotton, 1984), the ovarian maturation of *P. japonicus* develops in two steps: the primary vitellogenesis (V1) and the secondary vitellogenesis (V2) (Laubier *et al.*, in prep.). In optimum environment (Laubier-Bonichon, 1978) as well as after the ablation of the eyestalks (Panouse, 1943) of penaeid prawns (Laubier *et al.*, in prep.; Santiago, 1977; Pudadera and Primavera, 1981), the realization of the secondary vitellogenesis evolves in a very short time and corresponds principally in a mass uptake of vitellogenin by endocytosis in the ovocytes (Meusy and Charniaux-Cotton, 1984). Zerbib (1976) described in depth the nature of yolk from ovocytes of the Amphipod *Orchestia gammarella* where he distinguished two kinds of accumulation: the first, from endogenous source, is of glycoproteinic nature and the second, from exogenous source, is of a lipoglycocarotenoproteinic nature.

Studies were carried out to determine the quantitative and qualitative variations of lipids during the ovogenesis of some crustaceans (Galois, 1983; Middleditch *et al.*, 1980; Read and Caulton, 1980; Teshima and Kanazawa, 1983). Among the dietary lipids studied, Kanazawa *et al.* (1971, 1985) showed that cholesterol was required for crustacean growth and survival. Furthermore, this group of animals was not able to synthesize sterols from ^{14}C-acetate (Teshima and Kanazawa, 1971). The cholesterol stored in the egg yolk, beside its role as a membrane constituent, was found to be the precursor of steroid hormones in crustaceans (Kanazawa and Teshima, 1971) and its presence in the oocyte may play an essential role during vitellogenesis (Blanchet-Tournier, 1982).

To investigate the possible role of cholesterol in maturation and reproduction of female penaeid shrimps, the present study was undertaken to ascertain the distribution and fate of injected ^{14}C-cholesterol during the primary vitellogenesis and after induced secondary vitellogenesis by bilateral eyestalk ablation of prepuberal (the prepuberal stage has been defined by Chim, 1983 and Laubier *et al.*, 1985) female prawns *P. japonicus*.

MATERIALS AND METHODS

48 prepuberal (as defined by Chim, 1983 and Laubier *et al.*, 1985) prawns, *Penaeus japonicus*, of 2-3 cm of cephalo-thoracic length (CL), were supplied from the Mitsui Norin Kaiyosangyo Co. All animals received an injection of 0.1 µCi ^{14}C-cholesterol diluted in 3 µl of 95% ethanol and were maintained in aquariums at 23-24 °C. Animals were injected close to the heart in the pericardial space, as all the products are pumped by the heart to the general circulatory system.

72 hours after injection of ^{14}C-cholesterol the prawns were separated in two groups. Bilateral eyestalk ablation was sequentially performed in one group within 48 hours; the animals of the second group were kept intact. Different samples of prawns were taken 24, 72, 96 and 192 hours following injection and sacrificed. For each sacrificed animal, the ovaries, the hepatopancreas, the muscle, the gut, the eyes and the carcass (exoskeleton, hypodermis, gills, fat body, appendage's muscle and ventral nervous chain) were dissected, dried with filter paper, weighted and frozen. Radioactivity was measured after combustion of samples with the automatic sample combustion system Aloka ASC-112 with a Beckman liquid scintillation counter LS-230 using a toluen solution of PPO (0.6%) and POPOP (0.04%) as scintillation fluid.

Statistical analysis

The mean weights of the ovaries from the different experimental groups are compared by a covariance analysis that takes into account the variation of the size of animals (Lison, 1958). Hence mean ovarian weight was adjusted for each cephalothoracic length.

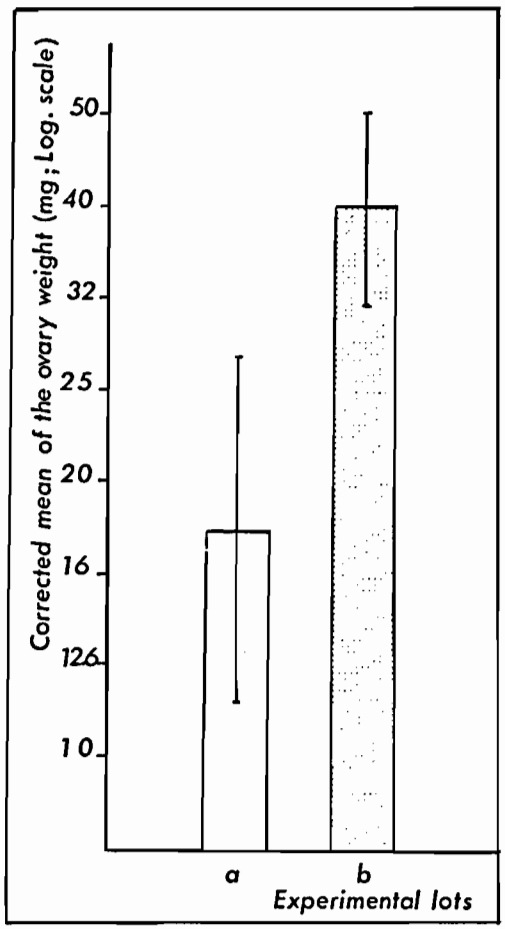

Figure 1. – Variations in the adjusted ovarian weight mean (logarithmic scale) for the same cephalothoracic length mean in the experimental series *a* and *b* (*a:* intact animals; *b:* bilateral eyestalk ablated animals four days after the ablation; $n = 6$ in each group).

RESULTS

Ablation of eyestalks

Figure 1 shows the mean weights of ovaries from intact animals and those deprived for 4 days of their eyestalks corrected for the size of the animals. The weight of ovaries of eyestalkess animals was significantly higher than those of intact animals ($p < 0.05$). This increase in ovarian weight observed in eyestalkless animals has been associated with an acceleration of primary vitellogenesis (V1) and the beginning of secondary vitellogenesis in the ovocyte (Laubier *et al.*, in prep.); the increase in total mass is due to an increase of intraovarian synthesis of vitellogenin but probably also to the uptake of vitellogenin from exogenous origin (Meusy and Charniaux-Cotton, 1984). Ovaries from intact animals showed only few ovocytes in primary vitellogenesis and none in secondary vitellogenesis.

Injection of ^{14}C-cholesterol

In *figure* 2, total incorporated radioactivity in whole animals before and after eyestalk ablation was studied at 24, 48, 72, 96 and 192 hours after ^{14}C-cholesterol injection. Whole body radioactivity remained constant throughout the experiment.

Figure 3 shows the incorporation of radioactivity into different organs and tissues from intact or eyestalk ablated animals, 192 hours after injection of ^{14}C-cholesterol. Results represent the concentration of label in each organ, expressed in terms of radioactivity (dpm)/mg of wet tissue or organ; radioactivity was incorporated in all tissues examined. For every organ studied, the comparison of radioactivity incorporated in intact and eyestalkless animals gave no

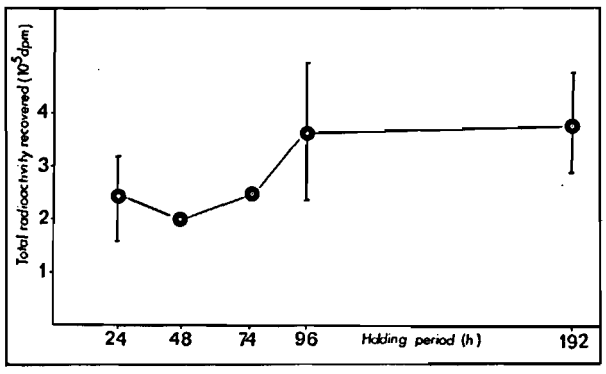

Figure 2. – Total body radioactivity of prepuberal female prawns recovered at different times following injection of ^{14}C-cholesterol ($n=6$ for 24, 96 and 192 hours).

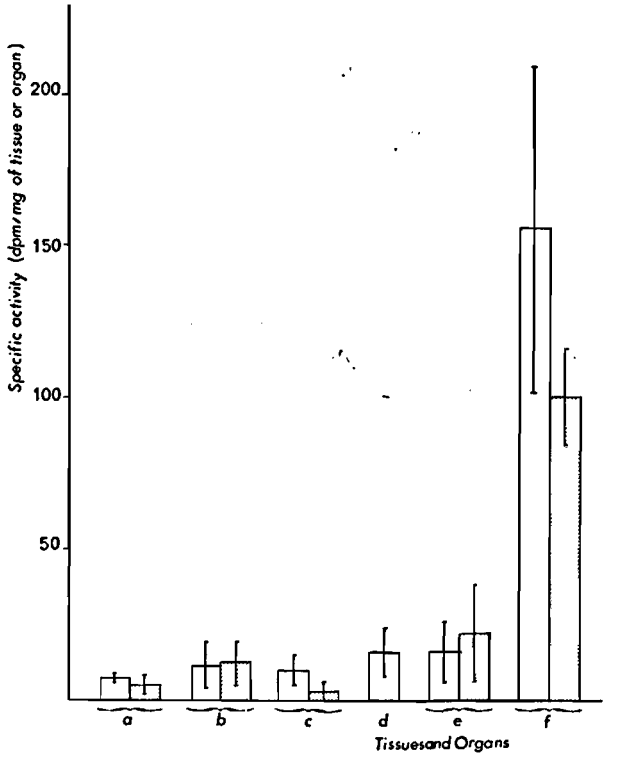

Figure 3. – Distribution of radioactivity in tissues and organs (dpm/mg tissue or organ) of prawns injected with ^{14}C-cholesterol 192 hours following the injection. ☐ intact animals. ▨ eyestalkless animals (*a:* carcass; *b:* muscle; *c:* gut; *d:* eyes and eyestalks; *e:* hepatopancreas; *f:* ovaries).

statistical difference. However, major difference of ^{14}C-cholesterol accumulation was observed in the ovaries when compared to other organs. In fact, the hepatopancreas, eyes and eyestalks, gut, muscle and carcass showed very little accumulation while in the ovaries radioactivity represented 6 to 40 times higher concentration.

The incorporation in different tissues of ^{14}C-cholesterol presumably under the form of cholesterol

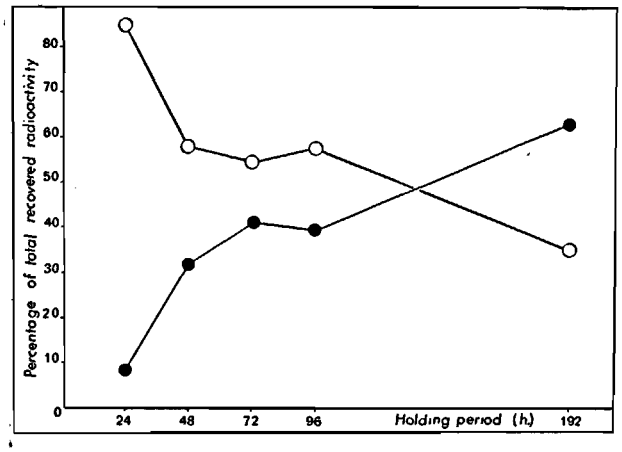

Figure 4. – Incorporation of radioactivity (%) into the carcass (○) and the muscle (●) following ^{14}C-cholesterol injection of intact prepuberal female prawns.

and/or its metabolites are shown in *figures* 4 and 5. Also, in *figure* 5, comparison of radioactivity found after 192 hours of ^{14}C-cholesterol injection in the ovary and hepatopancreas, from intact and eyestalkless animals, is depicted. The results presented herein represent the relative incorporation in each organ or tissue, in relation with total radioactivity found in the whole animal, expressed as percentage of organ or tissue radioactivity/total radioactivity recovered × 100. Hence, they reflect mobilization of the ^{14}C-labelled compounds among the different compartments independent of their respective volume of distribution. As expected, the carcass and muscle had the highest capacity for ^{14}C-cholesterol incorporation (*fig.* 4). Maximum accumulation in the carcass was achieved as early as 24 hours while the muscle presented a slow progressive accumulation. Interestingly, the increase in time of ^{14}C content in muscle (from 10 to 60% incorporation) was inversely correlated with the concomitant decrease from 85 to 40% observed in the carcass. The other organs studied, hepatopancreas, ovaries, eyes and eyestalks and gut (*fig.* 5) showed a relatively small incorporation of label. Minimal variations were not significant for the gut and eyes and eyestalk incorporation while hepatopancreas and ovary had their maximum accumulation early, followed by a significant decrease in contents with time. Comparison of radioactivity present in ovaries and hepatopancreas from intact and eyestalkless animals 192 hours after ^{14}C-cholesterol injection, showed a higher retention of label in eyestalkless animals that nevertheless was not statistically significant. At least in the ovaries, this may be explained by the increase

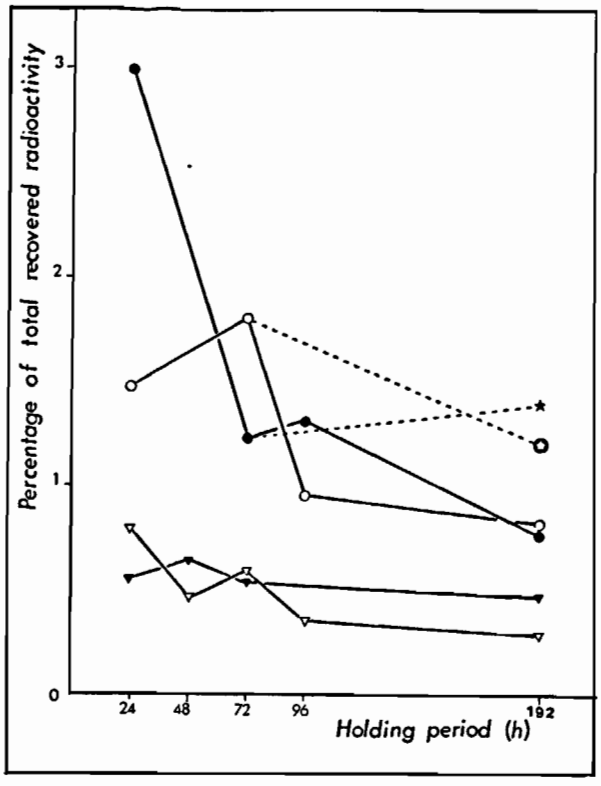

Figure 5. — Incorporation of ^{14}C-cholesterol (percentage of total incorporation) into the hepatopancreas (●), ovaries (○), eyes and eyestalks (▼) and gut (▽) from prepuberal intact animals and (☆, ✪) eyestalkless animals.

in weight observed after eyestalk ablation as seen in *figure* 1.

DISCUSSION

During our previous study, we have described the morphogenesis of the ovaries and recognized three stages in the penaeid prawns: juvenile, prepuberal and puberal stages (Laubier *et al.*, in prep.). The removal of the two eyestalks at these different stages led to an acceleration of vitellogenesis with an anticipated occurrence of secondary vitellogenesis in prepuberal prawns. These phenomena may be estimated through the study of the ovarian weights that are significantly higher in eyestalkless versus intact animals. The mechanism of induced secondary vitellogenesis or ovarian maturation and its hormonal control have been subject of a discussion in another study (Laubier *et al.*, in prep.).

During the occurrence of ovarian maturation (secondary vitellogenesis), large amounts of lipids are required fot the elaboration of egg yolk (Middleditch *et al.*, 1980; Read and Caulton, 1980; Teshima and Kanazawa, 1983). Free sterols are one of the major lipid class in the ovaries of penaeid prawn and represent 6.4 to 22% of total lipids (Teshima and Kanazawa, 1983). Among sterols, cholesterol was found to be the most predominant (Middleditch *et al.*, 1980) and to be essential for normal growth of marine crustaceans (Kanazawa *et al.*, 1971). Furthermore, marine crustaceans were not able to synthesize *de novo* the cholesterol molecule (Teshima and Kanazawa, 1971) and therefore, cholesterol found in penaeid eggs is likely to originate from two possible sources: the residual cholesterol present in the female ovary and the one that is ingested in the diet.

In this study, the use of animals with 5 g in total weight in studies of ovarian maturation represents a real advantage because small prawns are easy to obtain and more resistant to manipulation (e. g. injection or eyestalk ablation). The radioactive cholesterol injected in prawns was detected in every tissue studied, 8 days following the injection, but was present in higher concentrations in the ovaries than in other tissues or organ. There was an apparent accumulation of injected ^{14}C-cholesterol and/or its metabolic products in the ovaries throughout primary vitellogenesis as seen in intact prepuberal females. During the induced ovarian maturation produced by eyestalk ablation, corresponding to an acceleration of primary vitellogenesis and initiation of secondary vitellogenesis, the level of accumulation of the injected radioactive compound was not altered.

We reported previously the *in vivo* conversion of cholesterol to steroid hormones in the spiny lobster *Panulirus japonicus* (Kanazawa and Teshima, 1971) therefore it is possible that cholesterol accumulated in the ovaries of prepuberal prawns *P. japonicus* is, to some extent, used as a precursor for sexual hormone synthesis. Also, cholesterol from the ovaries can generate ecdysteroid hormone as suggested from its presence in ovaries of the crab *Carcinus maenas* (Lachaise and Hoffman, 1977). In fact, the role of ecdysteroids in crustaceans ovogenesis has been demonstrated (Blanchet-Tournier, 1982) while wether or not steroid sexual hormones play any role in the ovogenesis remains unsettled. Dietary cholesterol accumulated in the ovaries of mature female prawn is used by the larvae for their growth during the vitellotrophic stage (nauplius); the larvae, indeed, were found to be unable to synthesize cholesterol from acetate (Teshima *et al.*, 1983).

The study of tissue distribution of radioactivity at different time points following the injection of ^{14}C-cholesterol did not show clearly a transport of this molecule from the hepatopancreas to gonads as it was suggested for certain protein metabolites by Ceccaldi and Martin (1969). Only the transport of radioactive compounds from the exoskeleton (including hypodermis) to muscle was observed. The reason for this transport remains obscure.

The results presented herein suggest the importance of cholesterol in the ovogenesis process. In aquaculture, caution should be exercised when formulating the food for the broodstock that must be well balanced in dietary sterols. Nevertheless, further biochemical and histoautoradiographic investigations are required to elucidate the role of cholesterol and other steroids in ovogenesis.

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